American Journal of Medical Genetics 69:166–168 (1997)

Brief Clinical Report

Prezygotic Origin of the Isochromosome 12p in Pallister-Killian Syndrome

Valérie Cormier-Daire, Martine Le Merrer, Nadine Gigarel, Nicole Morichon, Marguerite Prieur, Stanislas Lyonnet, Michel Vekemans, and Arnold Munnich*

Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U. 393 et Département de Génétique, Hôpital des Enfants Malades, Paris, France

Pallister-Killian syndrome is a rare disorder comprising multiple congenital anomalies, streaks of hypo(hyper)pigmentation, seizures, profound mental retardation, and the presence of an extra metacentric chromosome i(12)(p10), usually limited to skin fibroblasts. The mechanism and parental origin of the extra chromosome i(12)(p10) are unknown. Here, we present a girl with Pallister-Killian syndrome and the i(12)(p10) in 50% of cultured skin fibroblasts. Using microsatellite DNA markers of chromosome 12p, we detected 3 alleles—including 2 different alleles of maternal origin—in cultured skin fibroblasts, suggesting that the tetrasomy 12p is the result of a prezygotic event, with a nondisjunction event during maternal meiosis. Am. J. Med. Genet. 69:166–168, 1997.

KEY WORDS: Pallister-Killian syndrome; isochrome 12p; prezygotic origin

INTRODUCTION

Pallister-Killian syndrome (PKS) is a rare sporadic form of mosaic tetrasomy 12p due to an extra metacentric chromosome i(12)(p10), usually detected in skin fibroblasts [Peltomäki et al., 1987; Warburton et al., 1987]. The clinical presentation of PKS includes profound mental retardation, seizures, streaks of hypo(hyper) pigmentation, and facial anomalies [Schinzel, 1991] including prominent forehead with sparse anterior scalp hair, flat occiput, hypertelorism, short nose with anteverted nostrils, flat nasal bridge, and short neck. The mechanism and parental origin of the extra chromosome i(12)(p10) are unknown, and the question of whether the rearranged chromosome 12 originates at meiosis or in the early divisions following the formation of the zygote has remained unanswered. Using microsatellite DNA markers of chromosome 12p, we observed maternal origin of the metacentric chromosome i(12)(p10) in a patient with PKS who further received one chromosome 12 from each parent. We were able to show that a prezygotic event with nondisjunction during maternal meiosis has occurred in the patient.

CLINICAL REPORT

A girl born to first-cousin, healthy parents at term (birth weight 4,100 g, length 54 cm, head circumference 36 cm) had poor head control at 5 months, seizures at 15 months, and was found to have a flat occiput with sparse anterior scalp hair, short neck, prominent forehead, hypertelorism, short nose with anteverted nostrils, and flat nasal bridge (Fig. 1). She also had streaks of hypo(hyper)pigmentation, short feet and hands, hip luxation, umbilical hernia, and a single palmar crease on the left hand.

METHODS

Chromosome analyses were performed on lymphocytes and fibroblasts derived from a skin biopsy specimen taken from a depigmented area of the left forearm, and chromosomes were studied from the initial culture.

To investigate whether polymorphic markers could help in detecting the origin of tetrasomy 12p, the inheritance pattern of microsatellite DNA markers of chromosomes 12 at loci D12S94, D12S99, D12S364, and D12S87 was tested in our patient and her parents. Genomic DNA (200 ng) was amplified in a final volume of 20 μl containing 1 μM unlabeled primer, 200 μM dNTPs, 1.25 mM $MgC1_2$, and 1 U Taq polymerase (Amersham) in the buffer recommended by the supplier. Samples were processed through 30 cycles (1

*Correspondence to: Arnold Munnich, Département de Génétique, Hôpital des Enfants Malades, 149, rue du Sèvres, 75743 Paris Cédex 15, France.

Received 22 January 1996; Accepted 20 May 1996

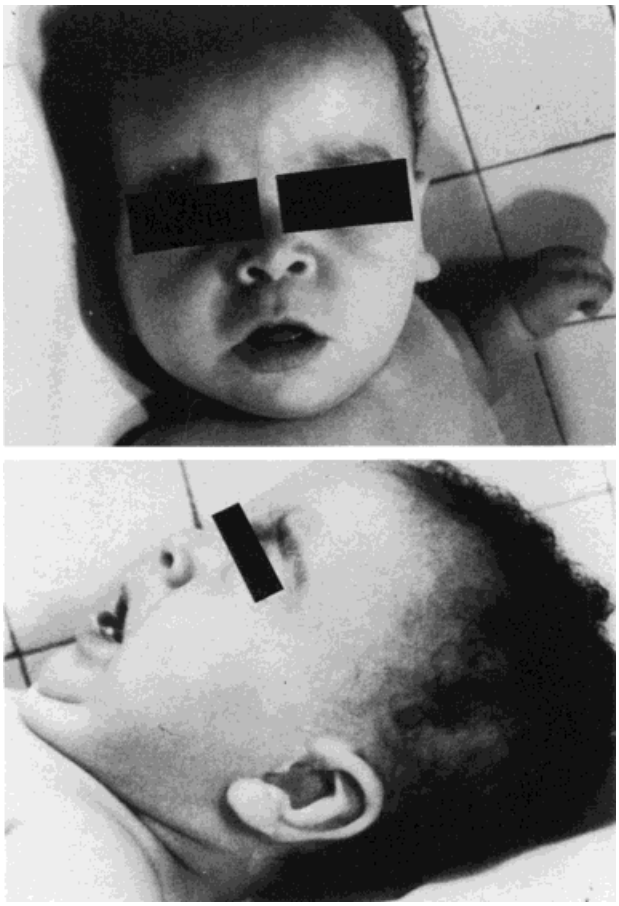

Fig. 1. Facial appearance in the Pallister-Killian syndrome. Note the flat occiput with sparse anterior scalp hair, prominent forehead, hypertelorism, short nose, and short neck.

minute at 94°C, 1 minute at 55°C, 1 minute at 72°C) and a final extension step of 10 minutes at 72°C. Polymerase chain reaction (PCR) products were incubated at 94°C for 5 minutes before loading (3 μl) on a 6% acrylamide, 7.5 M urea gel. Electrophoresis was performed at 1400 V, 65 W for 3 hours at 50°C. The gels were blotted onto nylon membranes (Appligene), labeled by chemiluminescence according to the manufacturer's instructions (ECL direct nucleic acid labeling and detection systems, Amersham Life Science), and exposed to X-ray film for 10 minutes.

RESULTS

Chromosome analysis of 100 lymphocyte mitoses showed an apparently normal female karyotype, but analysis of skin fibroblasts showed 50% of cells having an additional isochromosome [mos 46,XX/47XX,+ i(12)(p10)].

Regular biparental inheritance at the 4 loci tested was detected in the patient's lymphocytes (Fig. 2). However, in cultured skin fibroblasts, molecular studies at locus D12S94 detected 3 alleles. The normal chromosomes were biparental, and a third allele ("b") was present in the tetrasomic cell line, which could have come from either the mother or the father because both parents carried this allele. The results from D12S99 alone indicated that at least one maternal allele ("b") present in the tetrasomic cell line was not present in the disomic cell line. Finally, molecular studies at loci D12S364 (not shown) and D12S87 detected 3 alleles, including 2 alleles of maternal origin (Fig. 2).

DISCUSSION

Theoretically, the abnormal chromosome could originate either at meiosis or during the early cleavage divisions following the formation of the zygote. The observation of 3 different alleles in the proposita supports the view that tetrasomy 12p was the result of a prezygotic event in our patient. Its mechanism might involve impaired division of the centromere during a premeiotic mitosis. Abnormal pairing between the isochromosome and the normal homologue at meiosis I could cause nondisjunction. The fusion of a 24,+ i(12)(p10) gamete with a normal germ line would lead to a 47,+ i(12) zygote [Rivera et al., 1986]. Alternatively, one could postulate a nondisjunction event during maternal meiosis, resulting in a trisomic cell line followed by de novo formation of an isochromosome 12p. A similar meiotic origin was established previously in other trisomy mosaics [Hassold, 1985]. In keeping with this, it is worth remembering that advanced maternal age was reported previously as a predisposing cause of meiotic nondisjunction in PKS and in other aneuploidy conditions [Wenger et al., 1990]. Additional cases are necessary to decide whether i(12)(p10) has a consistently maternal origin in PKS.

Whatever the mechanism, the chromosomally normal cell line in PKS probably arises after conception from an aneuploid zygote. The secondary loss of the isochromosome suggests instability of i(12)(p10) during early embryogenesis, especially as the size reduction of the centromere probably contributes to the instability of the isochromosome during mitotic segregation. Finally, why cytogenetic and molecular techniques fail to detect the extra metacentric chromosome i(12)(p10) in lymphocytes may be related to somatic selection against cells containing the isochromosome in vivo in the blood and in vitro in fibroblast cultures or to a tendency to lose the extra chromosome with increasing cell divisions because of its instability [Hunter et al., 1985; Wenger et al., 1990].

In conclusion, microsatellite DNA markers of the 12p chromosome represent a simple and rapid tool for diagnosis of PKS. Additional molecular studies will help to decide whether the maternal origin of i(12)(p10) is a consistent finding in PKS.

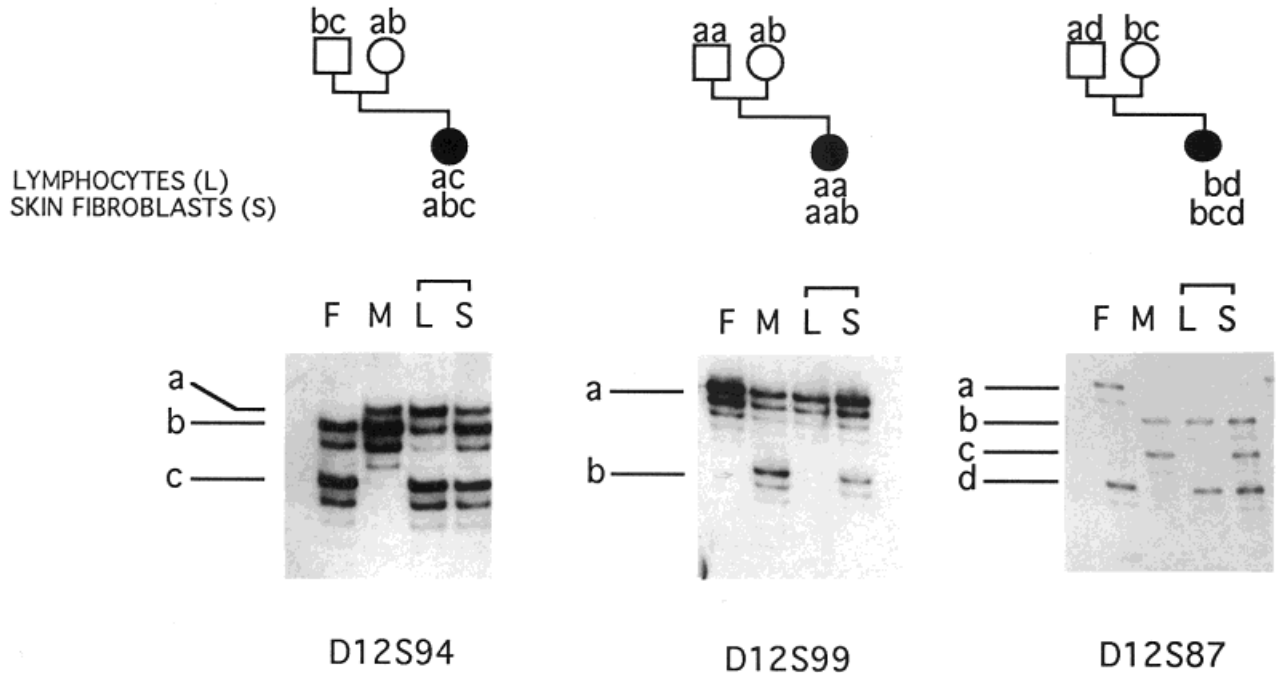

Fig. 2. Microsatellite DNA marker analyses at loci D12S94, D12S99, and D12S87. The genetic order 12pter-D12S94-D12S99-D12S87-12cen was established previously by analysis of CEPH reference families. Genomic DNA was extracted from blood lymphocytes and skin fibroblasts and amplified using markers AFM 206ze5, AFM 217xa7, and AFM 135xe3 at loci D12S94, D12S99, and D12S87, respectively. F, father's lymphocytes; M, mother's lymphocytes; L, S, proband's lymphocytes and skin fibroblasts.

REFERENCES

Hassold T (1985): The origin of aneuploidy in humans. In Vaughn-Dellarco V, Voytek P, Hollaender A (eds): "Aneuploidy: Etiology and Mechanisms." New York: Plenum Press, pp 103–116.

Hunter A, Clifford B, Cox DM (1985): The characteristic physiognomy and tissue specific karyotype distribution in the Pallister-Killian syndrome. Clin Genet 28:47–53.

Peltomäki P, Knuutila S, Ritvanen A, Kaitila I, De la Chapelle A (1987): Pallister-Killian syndrome: Cytogenetic and molecular studies. Clin Genet 31:399–405.

Rivera H, Rivas F, Cantú JH (1986): On the origin of extra chromosomes. Clin Genet 29:540–541.

Schinzel A (1991): Tetrasomy 12p (Pallister-Killian syndrome). J Med Genet 28:122–125.

Warburton D, Anyane-Yeboa K, Francke U (1987): Mosaic tetrasomy 12p: Four new cases and confirmation of the chromosomal origin of the supernumary chromosome in one of the Pallister-mosaic syndrome cases. Am J Med Genet 27:275–283.

Wenger S, Boone L, Steele M (1990): Mosaicism in Pallister i(12)(p10) syndrome. Am J Med Genet 35:523–525.